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C4 61. An expressed protein having a C-terminal thioester made
according to claim 59.

add DD
A marked-up version of the claims is attached hereto.

REMARKS

Claims 1-30 are pending and new claims 31-61 have been added. The Examiner has allowed claims 3-6, 10-13, 16-27, 29 and 30 where claims 3-6 and 10-13 are allowed subject to allowability of independent claims 1 and 15 and has rejected claims 1,2,7-9, 14, 15, 24 and 28. Applicants thank the Examiner for issuing a second office action in place of a Final Office Action as confirmed in a telephone conversation with the Examiner on May 29, 2002.

Applicants submit a 1.132 Declaration by Shaorong Chong as the sole inventor of claims 24 and claim 28 thereby rendering the rejection of these claims based on the June 1998 Chong publication moot.

Claims 1-3 have been amended and new claims 31-61 have been added. No new subject matter is believed to have been incorporated.

Support for amendments are as follows: for claim 1, on page 9, and "at least one" is consistent with present claim 8, for claim 31, on page 4, for claim 32 on page 11, for claim 33 on page 5, for claims 34 and 35 on page 9 for claim 36 and 54 on page 10, for claims 37, 38, 55 and 56 on page 13, for claims 39, 40 and 57, throughout the Application for example

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pages 5, 9, 12 and 13 and in the Examples, for claim 42, on page 3, line 25-page 4, line 18, for claim 58, on page 11, for claim 59 on page, for claim 60 on and for claim 61 on page 11 and Example 2.

Applicants thank the Examiner and his Supervisor for the courtesy of an interview on June 20, 2002 at the United States Patent Office. The subject matter of claims 1-3 and claim 42 were discussed in detail. The claims have been amended accordingly. Claim 1 has been amended to incorporate the Examiner's suggestions and thereby more distinctly describe the invention and is now believed to be allowable. Consequently all claims dependent off claim 1 (claims 2-7 and 15) are believed to be allowable. Applicants have added additional claims 31- 36 which are dependent from claim 1. Applicants have added claims 37-39 which are dependent from claim 8. Claim 40 and 42 claim the patentable subject matter of claim 1 in a different manner. Claims 43-57 provide equivalent dependencies from claim 42 as provided for claims 2-7 and 31-39. Claim 58 is directed to a novel method for generating a protein or peptide having a specified N-terminal amino acid, claim 59 is directed to a novel method for obtaining an expressed protein with a C-terminal thioester. Claim 61 is directed to an expressed protein having a C-terminal thioester.

In the interview, the Examiner and the Supervisory Examiner agreed with Applicants that the cited prior art references describing a chemical synthesis procedure for generating a peptide with a C-terminal thioester as described by Cannes, Kent, Tam, Offord and Hiatt are patentably distinct from the present claimed invention as described in

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claim 1 with particular reference to element (a), claim 40 and claim 42 and as such are not valid references under 35 U.S.C. §§ 102(e) and 103.

In the interview, the Examiner and the Supervisory Examiner additionally agreed with Applicants that transplicing is patentably distinct from the present claimed invention and that the Mills reference is not a valid prior art reference under 35 U.S.C. §103 alone or in combination with Canne, Tam or Kent references. It was agreed that even if all the above references were combined despite the absence of any motivation to do so, the teaching of the combination would not suggest, with "a reasonable expectation of success", to one of ordinary skill in the art, the present claimed invention. Examiner is therefore respectfully requested to reverse the rejection to claims 2, 7, 8, 9 under 35 U.S.C. §103.

Claim 8 and 9 are allowable over the cited prior art for reasons discussed in the Examiner's interview that include the requirement in claim 8(c) for "generating at least one C-terminal thioester tagged first protein from said plasmid of step (a)". Claim 24 is allowable over the cited art for reasons that include the lack of any suggestion in Mills that cleavage should be controllable for the purpose of producing a specified residue at the N-terminal of an adjacent protein. The autocatalytic transplicing described by Mills cleaves and splices without producing N-terminal intermediates. The cited art by Cannes, Tam, Kent, Offert and Hiatt do not suggest intein cleavage for generating a specified N-terminal.

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CONCLUSION


For the reasons set forth above, Applicants respectfully submit that the rejections set forth in the Official Action of April 23, 2002 have been overcome and that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned Attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned collect at the number shown below.

Respectfully submitted,

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MARKED-UP VERSION OF CLAIMS

1. (amended) A method for [fusion of] ligating a first expressed protein[s] with a second protein, the method comprising the steps of:

- (a) generating [at least one] a first target protein having a C-terminal thioester [tagged first], wherein the target protein is expressed in a host cell;
- (b) generating [at least one] a second target protein having a specified N-terminal; [and]
- (c) combining the first target protein with the second target protein in a mixture; and
- (d) [(c)] ligating [said] the first and [said] the second target protein[s] in the mixture.

2. (amended) The method of claim 1, wherein said first target protein of step (a) is generated from a first plasmid which further comprises [comprising] at least one nucleic acid sequence that encodes [a] at least one first intein having N-terminal cleavage activity and said second target protein of step (b) is generated from a second plasmid [comprising] which further comprises at least one nucleic acid sequence that encodes [a] at least one second intein having C-terminal activity.

3. (amended) The method of claim 2, wherein said at least one first intein comprises a first modified *Mth* RIR1 intein and wherein said at least one second [modified] intein comprises a second modified *Mth* RIR1 intein.

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31. (new) A method according to claim 2, wherein at least one first or at least one second intein may be an unmodified or a modified form of a naturally occurring intein.

32. (new) A method according to claim 1, wherein the C-terminal thioester of step (a) is formed in the presence of a thiol reagent.

33. (new) The method of claim 32, the thiol reagent is 2-mercaptoethanesulfonic acid.

34. (new) The method of claim 3 further comprising:
replacing in the first intein, a terminal proline residue with an alanine residue, the alanine residue having an N-terminal position with respect to a first amino-acid of the intein.

35. (new) The method of claim 3, further comprising:
replacing a C-terminal asparagine or cysteine of the intein with an alanine.

36. (new) The method of claim 2, wherein the first and second plasmids are capable of expression in at least one cell type selected from the group consisting of a bacterial, yeast, plant, insect and mammalian cell type.

37. (new) The method of claim 8, wherein step (b) further comprises cleaving of an intein controllably, or by induction using a nucleophilic compound.

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38. (new) The method of claim 37, wherein the nucleophilic compound is a thiol reagent.
39. (new) The method of claim 37, wherein controlling cleavage of the intein includes modulating temperature, pH, salt, chaotropic agents, or any combinations thereof.
40. (new) A method for ligating a first protein target to a second target protein, comprising:
- (a) applying means for generating fusion proteins of the first protein and at least one first intein and a second protein and at least one second intein where the first intein and the second intein may be the same or different;
 - (b) applying means for cleaving the first and the second fusion protein so as to provide a C-terminal thioester on one target protein and a specified N-terminal on the second target protein; and
 - (c) applying means for permitting the first target protein to ligate to the second target protein.
41. (new) A method according to claim 40, wherein step (b) further comprises applying means for separating the first and second target proteins from the cleaved inteins.
42. (new) A method for obtaining a protein product formed from two target proteins, said method comprising the steps of:

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- (a) generating a first target protein fused to at least one first intein and a second target protein fused to at least one second intein, wherein the first intein may be the same or different from the second intein;
- (b) cleaving the first target protein from at least one first intein so as to form a C-terminal thioester; and cleaving the second target protein from at least one second intein so as to provide a specified N-terminal; and
- (c) ligating the first target protein with the second target protein to form the protein product.

43. (new) The method of claim 42, wherein the first target protein of step (a) is generated from a first plasmid which further comprises at least one nucleic acid sequence that encodes the at least one first intein and said second target protein of step (a) is expressed by a second plasmid which further comprises at least one nucleic acid sequence that encodes the at least one second intein.

44. (new) The method of claim 43, wherein the first intein comprises a first modified *Mth* RIR1 intein and wherein the second intein comprises a second modified *Mth* RIR1 intein.

45. (new) The method of claim 44, wherein the first modified *Mth* RIR1 intein is selected from the group consisting of a Pro⁻¹ to Gly mutant intein, and a Pro⁻¹ to Asn¹³⁴ to Gly-Ala mutant intein, and wherein said modified *Mth* RIR1 intein is selected from the group consisting of a Pro⁻¹ to Cys¹ to Gly-Ser mutant intein and a Pro⁻¹-Cys¹ to Gly-Ala mutant intein.

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46. (new) The method of claim 43, wherein the first plasmid is selected from the group consisting of pMRB8A, pMRB8G1 and pMRB9GA and pBRL-A and wherein the second plasmid is selected from the group consisting of PMRB9GS, pMRB9GA and pBRL-A.

47. (new) The method of claim 44, wherein the first target protein of step (a) is generated by a thiol reagent-induced cleavage product of said first modified Mth RIR1 intein and said second target protein of step (a) is generated by temperature and/or pH induced cleavage of said second modified Mth RIR1 intein.

48. (new) The method of claim 43, wherein the specified N-terminal comprises a cysteine.

49. (new) A method according to claim 43, wherein at least one first or at least one second intein may be an unmodified or a modified form of a naturally occurring intein.

50. (new) A method according to claim 42, wherein the C-terminal thioester of step (b) is formed in the presence of a thiol reagent.

51. (new) The method of claim 50, the thiol reagent is 2-mercaptoethanesulfonic acid.

52. (new) The method of claim 44, further comprising:

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replacing in the first intein, a terminal proline residue with an alanine residue, the alanine residue having an N-terminal position with respect to a first amino acid of the intein.

53. (new) The method of claim 44, further comprising:
replacing a C-terminal asparagine or cysteine of the intein by an alanine.

54. (new) The method of claim 43, wherein the first and second plasmids are capable of expression in at least one cell type selected from the group consisting of a bacterial, plant, insect and mammalian cell type.

55. (new) The method of claim 49, wherein step (b) further comprises:
cleaving of an intein controllably, or by induction using a nucleophilic compound.

56. (new) The method of claim 55, wherein the nucleophilic compound is a thiol reagents.

57. (new) The method of claim 55, wherein controlling cleavage of the intein includes modulating temperature, pH, salt, chaotropic agents, or any combinations thereof.

58. (new) A method for generating a protein or peptide having a specified N-terminal amino acid, comprising:

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obtaining a nucleic acid encoding the protein or peptide having an intein coding sequence adjacent to a specified amino acid codon of the target protein;

causing the nucleic acid product to be expressed; and

cleaving the intein from the expressed nucleic acid product so as to generate the protein or peptide with the specified N-terminal amino acid.

59. (new) A method for obtaining an expressed protein with a C-terminal thioester, comprising:

- (a) obtaining the expressed precursor protein, the precursor having an intein; and
- (b) reacting the precursor protein with a thiol reagent so as (i) to remove the cleavage element and (ii) to obtain the expressed protein with the C-terminal thioester.

60. (new) The method of claim 59, wherein the intein is an *Mth* RIR1 intein.

61. (new) An expressed protein having a C-terminal thioester made according to claim 59.